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Maternal vitamin D and markers of glycaemia during pregnancy in the Belfast centre of the Hyperglycaemia and Adverse Pregnancy Outcome study.

**Running title:** Vitamin D and glycaemia during pregnancy

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**Novelty statement:**

- This is a large scale observational study reporting serum vitamin D concentrations in a pregnant population in Northern Ireland.
- The study shows a high level of vitamin D deficiency in a white European cohort at 55°N.

- The current study suggests that vitamin D and glucose metabolism in pregnancy are not linked.

## Abstract

**Aims-** The aim of the present study was to measure total 25-hydroxyvitamin D (25OHD) in women mid-pregnancy in the Belfast centre of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) observational study and investigate the associations between 25OHD and markers of gestational diabetes mellitus (GDM) and lipid biomarkers.

**Methods-** 1585 pregnant women had serum samples available for measurement. Participants were recruited from the Royal Jubilee Maternity Hospital, Belfast, Northern Ireland between 24-32 weeks gestation as part of the HAPO study. 25OHD concentrations were measured by liquid chromatography tandem mass spectrometry. Glucose, C-peptide and lipids were previously analysed in a central laboratory. Statistical analysis was performed.

**Results-** The median (interquartile range) 25OHD concentration during pregnancy was 38.6 (24.1 to 60.7) nmol/L, with 65.8% of women being vitamin D deficient ( $\leq 50$  nmol/L). In regression analysis, the association between maternal 25OHD and fasting plasma glucose approached significance [regression coefficient (95% CI) -0.017(-0.034 to 0.001);  $p=0.06$ ], and a significant positive association was observed between maternal 25OHD and beta-cell function [1.013 (1.001 to 1.024);  $p=0.031$ ]. Maternal 25OHD was positively associated with HDL [0.047 (0.021 to 0.073)  $p \leq 0.001$ ] and total cholesterol [0.085 (0.002 to 0.167);  $p=0.044$ ] in regression analysis.

**Conclusions -** These results indicate a high prevalence of vitamin D deficiency during pregnancy which requires identification and treatment. However, only weak associations were observed between 25OHD and markers of glucose and insulin metabolism. This would suggest that these are of doubtful clinical significance.

**Keywords:** nutrition and diet, pregnancy, insulin sensitivity, gestational diabetes mellitus, lipid metabolism

## Introduction

Vitamin D sufficiency during pregnancy is of the utmost importance as vitamin D diffuses across the placenta and is transferred to the neonate; as a result maternal deficiency will translate into neonatal deficiency (1). A systematic review of the global prevalence of deficiency (defined as  $< 50$  nmol/l) in pregnant women found vitamin D deficiency varied greatly across Europe with incidence rates ranging from 18-90% (2). At present, there is a lack of data on large cohorts on the vitamin D status of healthy pregnant women in Northern Ireland; this makes it difficult to develop recommendations for achieving optimal 25-hydroxyvitamin D (25OHD) status in pregnancy.

Pregnancy is associated with altered glucose metabolism, an increase in insulin resistance and a decrease in insulin sensitivity, and changes in lipid metabolism (3). The regular function of vitamin D is to maintain calcium-phosphate homeostasis (4), however, there is increasing evidence for a possible beneficial impact of vitamin D sufficiency on glycaemia in pregnancy (5,6). Gestational Diabetes Mellitus (GDM) is defined as glucose intolerance with onset or first recognition during pregnancy, as a result of pregnancy associated insulin resistance and impaired insulin secretion. It occurs in approximately 2-13% of all pregnancies depending on diagnostic criteria and the population being studied (7,8). Women with GDM have an increased risk of adverse pregnancy outcomes and subsequent type 2 diabetes after pregnancy (7). A recent meta-analysis of observational studies reported that vitamin D deficiency was associated with a significant increased risk for the development of GDM (6,9). However, a meta-analysis of randomised controlled trials (RCT) found no difference in the incidence of GDM among women in the control group vs vitamin D supplement group (10). The impact of vitamin D deficiency on GDM, therefore needs clarification as treatment with 25OHD potentially offers an inexpensive solution to reduce the frequency of GDM.

The aim of this study was to measure 25OHD<sub>2</sub>/D<sub>3</sub> and dietary vitamin D in the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) cohort of pregnant women at 24-32 weeks gestation in the Belfast Centre, and to investigate the association of 25OHD with markers of glucose, insulin resistance, beta-cell function and lipids of these women.

## **Participants and methods**

Details of the methodology of the HAPO study have been published elsewhere (11,12). Briefly, the HAPO study was a 15-centre multicultural and multinational study designed to examine the association between maternal hyperglycaemia, and adverse pregnancy outcomes with singleton pregnancies whose results on OGTT were below the thresholds for diabetes. All pregnant women at a given centre were eligible to participate. Each participant underwent a standard 75g OGTT between 24-32 weeks gestation (optimal-28 weeks), with sampling of fasting plasma glucose and at one hour and two hours (glucose assays: Vitros 750; Ortho-Clinical Diagnostics, Raritan, NJ, USA; interassay coefficient of variation 4.4%). OGTT results were blinded to the clinician responsible for the care of the pregnant woman unless the fasting plasma glucose level exceeded 5.8 mmol/L or the 2-hour post-load level exceeded 11.1 mmol/L. Additional blood samples were collected concurrently for storage and future biomarker analysis.

Maternal height, weight and blood pressure were measured at the OGTT and data concerning maternal smoking habits, and alcohol use were collected using a standardised questionnaire at this visit. In addition, participants at the OGTT completed a semi-quantitative validated food-frequency questionnaire (FFQ) which was used to assess usual dietary intake (13). Mean dietary vitamin D intake was calculated from the FFQ using the nutritional software package Q-Builder (Questionnaire Design System), version 2.0 (Tinuviel Software, Anglesey, UK) which uses UK food composition tables to quantify nutrient intakes. Quantification of dietary intake of vitamin D was based on food sources alone, as the FFQ was not designed to ascertain the quantification of vitamin D entering the diet via food fortification or vitamin supplementation.

25OHD<sub>2</sub>/D<sub>3</sub> was measured in serum using a liquid chromatography tandem-mass spectrometry (LC-MS/MS) method [Waters® Xevo TQ-S® & ACQUITY UPLC (Waters, Elstree, UK)]. The inter-assay CVs of the method for 25OHD<sub>2</sub> and 25OHD<sub>3</sub> were 4.4% and 3.4% at concentration 16.1 nmol/L, respectively, while the intra-assay CVs were 2.7 and 2.3%, respectively. The quality and accuracy of serum 25OHD analysis using the LC-MS/MS method in our laboratory was monitored on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (Charing Cross Hospital). C-peptide was measured only in non-haemolysed samples by a two-way immunometric assay on an Autodelphia instrument (Waltham, MA). Lipids were also measured on the Cobas C8000 analyser using the enzymatic colorimetric method. The intra-assay CVs for all lipids were <2.5%, and the inter-assay CVs for all lipids were <2.8%. LDL was derived using the Friedewald equation if triglyceride levels did not exceed 4.52 mmol/L. HOMA-IR and HOMA-beta were calculated using the HOMA2 calculator (14). C-peptide was used in the calculation of HOMA-IR and HOMA-beta as opposed to insulin as it is more stable due to haemolysis. In addition, a previous publication on the HAPO study have used c-peptide in their calculations of HOMA (15).

Overall, 23316 blinded participants successfully completed the HAPO study. Of the participating 1677 women from the Belfast centre, 37 women were excluded from the study due to OGTT glucose levels exceeding predefined thresholds leading to unblinding of the caregivers and treatment of the participants [n=1640 (98%)]. A further 28 women who were of non-white European ethnicity were also excluded due to the relationship between GDM and ethnicity (16); this resulted in 1612 (96%) of the cohort being available for study. Serological samples for the measurement of vitamin D were available for 1585 women.



Written informed consent was obtained from all study participants. Ethical approval was obtained from the relevant ethics committee and the research adhered to the tenets of the Declaration of Helsinki.

### Statistical Analysis

Statistical analysis was carried out using SPSS version 21 (IBM Corp, Armonk, NY, USA). Variables were examined using normality plots and serum 25OHD, serum triglyceride, HOMA-IR, and HOMA-beta were logarithmically transformed to the base 2 as their distributions were positively skewed. Season of maternal OGTT was defined as winter/spring (December, January, February, March, April, May) or summer/autumn (June, July, August, September, October, November). Total 25OHD was composed of 25OHD<sub>2</sub> and 25OHD<sub>3</sub>, of which 25OHD<sub>3</sub> was the main constituent. Vitamin D deficiency in this cohort was defined as  $\leq 50$  nmol/L as per Institute of Medicine guidelines(17).

Descriptive statistics are presented for the cohort's continuous demographic and biochemical variables as mean  $\pm$  standard deviation (SD) for normally distributed variables, median and interquartile range (IQR) for non-normally distributed variables, and n (%) were used to describe the cohort's categorical variables.

Independent sample t-tests and chi-square tests were used to compare participants who were vitamin D deficient and sufficient for continuous and categorical variables, respectively. Results are presented as mean  $\pm$  SD for normally distributed variables and geometric mean  $\pm$  SD for log-transformed variables. Pearson correlation coefficients were used to assess the association between total 25OHD concentrations and continuous variables.

Multiple linear regression analysis was used to determine if circulating total 25OHD had an independent association with maternal fasting, 1-hour and 2-hours plasma glucose, HOMA-IR, HOMA-beta, cholesterol, LDL, HDL and triglycerides controlled for confounding

variables. Binary logistic regression was performed to determine if maternal total 25OHD had an independent association with GDM [as defined by the 2013, IADPSG/WHO criteria (18)].

Variables included in the multiple regression analysis were chosen based on previous literature and prior bivariate results from this cohort. Maternal age at OGTT, maternal height, BMI at OGTT, gestational age at OGTT, smoker during pregnancy and family history of diabetes were identified based on similar literature and analysis (5,19). In addition, season of sampling and maternal education (years of education) were also included as a result of bivariate analysis. Regression coefficients were back transformed if the dependent variable was log transformed. A p value less than or equal to 0.05 was considered statistically significant.

## Results

Table 1 shows the clinical and biochemical characteristics of mothers in the Belfast HAPO study. The mean  $\pm$  SD age (n=1585) and BMI (n=1584) at the OGTT were  $29.7 \pm 5.5$  years (n=1612), and  $28.3 \pm 4.6$  kg/m<sup>2</sup>, respectively. Women underwent blood testing at a mean  $\pm$ SD of  $29.0 \pm 1.2$  weeks gestation (n=1585). The prevalence of cigarette smoking and alcohol consumption was relatively high (24.1 and 26.9%, respectively). Fasting, 1-hour and 2-hour mean  $\pm$  SD plasma glucose levels were  $4.6 \pm 0.3$  mmol/L (n=1585),  $7.4 \pm 1.6$  mmol/L (n=1584) and  $6.0 \pm 1.1$  mmol/L (n=1585), respectively.

The median and IQR of maternal 25OHD concentration was 38.6 (24.1 to 60.7) nmol/L (n=1585). No 3-epi-25OHD<sub>2</sub> was detectable in any of the samples. Mean 3-epi-25OHD<sub>3</sub> levels were low [ $2.9 \pm 1.9$  nmol/L (n=1512)] and present in 95% of all participants sampled. Dietary vitamin D from diet alone was low [ $3.3 \pm 2.5$   $\mu$ g/day (n=1541)]. However, it should be noted that the FFQ used in the Belfast HAPO study was not designed to quantify vitamin supplementation or fortified food use. The prevalence of vitamin D deficiency was high, with 65.8% of women having 25OHD levels less than or equal to 50 nmol/L.

Table 2 compares clinical and biochemical characteristics by vitamin D deficiency ( $\leq 50$  nmol/L versus  $>50$  nmol/L). A significantly higher proportion of smokers during pregnancy were vitamin D deficient ( $p \leq 0.001$ ). There was also statistically significant evidence of seasonal variation. A higher proportion of women who were sufficient in vitamin D were sampled in summer and autumn, whereas, a higher percentage of women deficient in vitamin D were sampled in winter & spring ( $p \leq 0.001$ ). Women who were vitamin D deficient were more likely to be younger and have a higher BMI than those women who were vitamin D sufficient. Women with vitamin D deficiency had significantly fewer years in education compared to those who were vitamin D sufficient. No statistically significant differences

were observed between women who were vitamin D deficient or sufficient in terms of markers of glucose and insulin metabolism. No statistically significant differences in women diagnosed with GDM were observed for those who were vitamin D deficient and sufficient (Table 2). In addition, when 25OHD was expressed as a continuous variable, no statistically significant difference was observed between those diagnosed with and without GDM ( $36.9 \pm 1.9$  and  $37.8 \pm 2.0$  nmol/L, respectively;  $p=0.61$ -data not shown).

Circulating levels of 25OHD were not significantly correlated with age, weight or height, however, 25OHD was significantly and positively correlated with length of education ( $p \leq 0.001$ ). The association between 25OHD and BMI at OGTT approached significance ( $p=0.060$ ) (data not shown).

The adjusted analysis involving 25OHD levels and fasting plasma glucose approached significance [regression coefficient (95% CI)  $-0.017(-0.034$  to  $0.001)$ ;  $p=0.06$ ] (Table 3). No association was found between one-hour post OGTT glucose and two-hour post OGTT glucose and 25OHD levels. A weak significant positive association was observed between 25OHD and HOMA- beta [ $1.013$  ( $1.001$  to  $1.024$ );  $p=0.031$ ] in adjusted analysis. A doubling of maternal 25OHD levels was associated with HOMA-beta higher by approximately 1%. No corresponding association was observed between 25OHD and HOMA-IR. In keeping with these results, no association was observed between vitamin D and GDM.

Weak significant positive associations were observed between vitamin D and both total cholesterol [ $0.085$  ( $0.002$  to  $0.167$ );  $p=0.044$ ] and HDL cholesterol [ $0.047$  ( $0.021$  to  $0.073$ )  $p \leq 0.001$ ] in adjusted analyses.

## Discussion

Our results have shown a relatively high prevalence of vitamin D deficiency (65.8%) in a northern hemisphere white European pregnant population. This may be due to the seasonal effect as well as the low intake of dietary vitamin D. We found little association with total 25OHD and traditional markers of GDM during pregnancy. Total 25OHD was associated with HOMA-beta but with no corresponding relationship with HOMA-IR. There was a weakly positive association between total 25OHD and both total cholesterol and HDL.

Vitamin D deficiency is a common disorder globally. A recent systematic review by Saraf and colleagues (2015) found that vitamin D deficiency (<50 nmol/L) varied around the world. Deficiency was present in 42-72% of pregnant women in the Americas, 18-90% in Europe, 46% in the Eastern Mediterranean, 66-96% in South-East Asia and 41-97% in the Western pacific region (1). The level of total 25OHD in the current study was lower than that observed in a similar cohort of pregnant women in the southwest of England [The Avon Longitudinal Study of Parents and Children (ALSPAC) study (n=3,960)], which reported a median circulating 25OHD level in the third trimester of 67.4 (IQR- 46.8-93.0) nmol/L (20). In addition, 34% of participants in that study in the third trimester had 25OHD levels less than 50 nmol/L (20). It is conceivable that the higher levels of vitamin D in the ALSPAC study relates to the higher sunshine levels in the southwest of England which receives 1,750 hours of sunshine annually, compared with 1,450 hours of annual sunshine in Belfast (21). This suggests there could be significant differences within the UK during pregnancy in terms of endogenous production of vitamin D<sub>3</sub>. In addition to the reduced production of vitamin D<sub>3</sub> in the Belfast HAPO cohort, the dietary intake of vitamin D was low. This might be due to the low consumption of fish (oily fish in particular). Participants in the Belfast HAPO cohort on average failed to meet the recommended nutrient intake (RNI) for vitamin D (10 µg/day)(22), however, this could be explained by the lack of information on vitamin D

derived from vitamin supplementation and fortified foods. Dietary vitamin D consumption was comparable to that reported in the latest UK National Diet and Nutrition Survey (NDNS)(23), where the average dietary vitamin D intake was 3.4 µg/day. This coupled with the seasonal effect observed in the current study suggests that pregnant, white European women in Northern Ireland may benefit from enhanced dietary and supplementary strategies to increase 25OHD levels to an optimal level and that existing approaches are insufficient.

In the current study, weak associations between 25OHD and glucose and insulin markers are in line with the lack of a significant association between 25OHD and GDM. Whitelaw and colleagues studied 596 white Europeans in England using a similar method of 25OHD analysis as the current study and reported similar results. They observed 66% of women were vitamin D deficient ( $\leq 50$  nmol/L), and found weak inverse associations between 25OHD and fasting plasma glucose (24).

A sub-group of the HAPO cohort at the Brisbane centre (n=399) performed a similar analysis to the current study, and on multivariate analysis found that 25OHD was significantly negatively associated with fasting plasma glucose [-0.047 (-0.084 to -0.010);p=0.012] , but not with 1 hour or 2-hour plasma glucose. In addition, a significant correlation was observed between total 25OHD and HOMA-beta (p=0.009) in bivariate analysis, but not with HOMA-IR. The Brisbane HAPO cohort however were vitamin D replete (mean 25OHD $\pm$  SD: 132.5  $\pm$  44.0 nmol/L), whereas, in the current study, the prevalence of vitamin D deficiency was 65.8% and mean 25OHD levels were <50 nmol/L. It was suggested by the Brisbane authors that the association observed in their study would also exist in populations who were vitamin D deficient (5). Our results partially confirm their hypothesis as we found a significant association with HOMA-beta, with no corresponding relationship with HOMA-IR in adjusted analysis. In addition, 25OHD had a borderline significant negative association with fasting plasma glucose. However, the associations found in the current study, were for the most part,

weak, compared with the Brisbane data. Our results are more similar to those by Josefson and colleagues who reported findings from a North American subset of HAPO (n=360), in which no significant associations were found between 25OHD and fasting plasma glucose or GDM among participants with a mean 25OHD vitamin D concentration of 93 nmol/l (25). The two studies described above, together with the current study all used a similar study protocol, laboratory methodology for 25OHD analysis, and they all employed the same diagnostic criteria for GDM, albeit with a spectrum of vitamin D concentrations. The inconsistent results from the three studies however support the need for further research and strengthen the case for a large scale RCT in pregnancy to clarify the role of 25OHD in the development of GDM.

The significant positive associations between 25OHD and total and HDL cholesterol could be a chance finding, although an increase in cholesterol levels during vitamin D deficiency is biologically plausible. For example, Al-Ajlan and colleagues performed a cross-sectional observational study to examine the effect of vitamin D deficiency on the lipid metabolic profile in Saudi women. They observed that serum vitamin D was positively associated with total serum cholesterol and triglyceride levels. The Saudi study population had a mean total 25OHD of 19 nmol/L. The authors suggested that the positive association between cholesterol and vitamin D was due to a combination of deficient vitamin D status with the high metabolic demands of pregnancy (26). Replication of these findings in a general population would be helpful to establish if the association is true, and if there is a level of vitamin D sufficiency (i.e. > 50 or > 75 nmol/l) above which vitamin D is negatively associated with serum cholesterol. A possible association between vitamin D and cardiovascular disease (CVD) is well documented in the literature (27–29). The combination of elevated levels of serum LDL cholesterol and decreased levels of HDL cholesterol increase the risk of atherosclerosis which leads to CVD (30). Therefore, any dietary intervention that increases HDL and lowers LDL cholesterol potentially might improve

cardiovascular health. It has been suggested that the association between HDL and 25OHD might be due to the stimulation of apolipoprotein A1 and/or apolipoprotein A5 by vitamin D<sub>3</sub> (31). However, this has yet to be confirmed. A recent study observed that 25OHD was associated with lower HDL and HDL was associated with lower 25OHD suggesting that the direction of the association between 25OHD and HDL may be bidirectional (32). It is possible that the positive association observed between 25OHD and HDL may simply be a marker of overall good health status. In addition, lipid metabolism is different during pregnancy, and the results from the current study may not be reflective in a general population. A number of reviews have been published on vitamin D and lipids in non-pregnant populations (33,34), and most of these found that 25OHD was positively associated with HDL cholesterol, and negatively associated with LDL.

The current study has several limitations. The study population was 100% white European, and with the variable rates of vitamin D requirements among different ethnic groups, the results cannot be applied to all population groups. Insulin resistance and beta cell function were estimated using the HOMA equations. These equations reflect only the fasting state and are less sensitive than direct measurements such as the hyperinsulinemic euglycemic clamp. The BMI used in the HAPO study was measured at 24-32 weeks gestation and it is possible that BMI at this stage in pregnancy may not be representative of the true BMI status of the mother. In addition, dietary intake was assessed using a FFQ, and not the gold standard method of a food diary. On the other hand, the strengths of this study include the large number of participants studied, the rigorous nature of the research methodology of the HAPO study including the ability to control for an extensive number of confounding variables, the state of the art measurement of maternal vitamin D and its metabolites and the homogeneity of the population.



In conclusion, this study demonstrates that vitamin D deficiency and a low dietary vitamin D intake are common in a large group of pregnant women in Belfast, UK. Weak associations were observed between vitamin D and HOMA-beta, while the association with fasting plasma glucose approached significance. Overall, this suggests that vitamin D does not have a role in the development of GDM during pregnancy and would concur with evolving data in the non-pregnant context (35,36). The positive association of 25OHD and HDL merits further investigation to confirm the association and to explore possible biological mechanisms.

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Table 1 Clinical and biochemical characteristics of Belfast HAPO participants at OGTT visit  
at an average of 28 weeks gestation.

Variable	N (%)	Range	Mean $\pm$ SD
Age (years)	1585	18.1 to 43.3	29.7 $\pm$ 5.5
Weight (kg)	1584	46.9 to 137.3	74.9 $\pm$ 12.7
Height (cm)	1585	139.8 to 183.1	162.9 $\pm$ 6.2
BMI at OGTT (kg/m <sup>2</sup> )	1584	18.1 to 50.3	28.3 $\pm$ 4.6
Gestation (weeks)	1585	23.9 to 33.1	29.0 $\pm$ 1.2
Ethnicity		-	-
White European	1585/1585 (100%)		
Smoker in pregnancy		-	-
Yes	382/1585 (24.1)		
Alcohol use in pregnancy		-	-
Yes	426/1585 (26.9%)		
Education (years)	1574	10 to 27	15 $\pm$ 3
Season of sampling			
Summer & Autumn	813/1585 (51.3)	-	-
Winter & Spring	772/1585 (48.7)		
Family history of diabetes		-	-
Yes	253/1574 (1.6%)		
Fasting plasma glucose (mmol/L)	1585	3.6 to 5.8	4.6 $\pm$ 0.3
1-hour OGTT plasma glucose (mmol/L)	1584	2.8 to 13.2	7.4 $\pm$ 1.6
2-hour OGTT plasma glucose (mmol/L)	1585	2.4 to 10.2	6.0 $\pm$ 1.1
HOMA-IR <sup>a</sup>	1569	0.4 to 5.0	1.3 (1.1 to 1.8)
HOMA-beta <sup>a</sup>	1569	61.3 to 379.6	141.9 (123.7 to 163.9)
Diagnosis of GDM			
Yes	243/1585 (15.3)	-	-
Cholesterol (mmol/L)	1480	0.05 to 11.9	6.5 $\pm$ 1.4
HDL-cholesterol (mmol/L)	1480	0.4 to 3.3	1.7 $\pm$ 0.4
LDL-cholesterol (mmol/L)	1454	0.6 to 9.4	3.8 $\pm$ 1.2
Triglycerides (mmol/L) <sup>a</sup>	1480	0.4 to 6.1	2.1 (1.7 to 2.6)
Total 25OHD (nmol/L) <sup>a</sup>	1585	3.1 to 266.1	38.6 (24.1 to 60.7)
3-epi-25OHD <sub>3</sub> (nmol/L)	1512	2.7 to 20.0	2.9 $\pm$ 1.9
Dietary vitamin D ( $\mu$ g/day)	1541	0.0 to 29.8	3.3 $\pm$ 2.5
Vitamin D deficiency ( $\leq$ 50 nmol/L)	1043/1585 (65.8%)	-	-

Continuous variable values are expressed as range and mean  $\pm$  SD and categorical variables are expressed as number and percentage.

a- Reported as median and interquartile range

Gestational diabetes defined retrospectively by IADPSG/WHO criteria

25OHD, 25-hydroxyvitamin D HAPO, Hyperglycaemia and Adverse Pregnancy Outcome GDM, Gestational Diabetes Mellitus



Table 2- Comparison of clinical and biochemical characteristics of Belfast HAPO participants at OGTT visit at an average of 28 weeks gestation by maternal vitamin D deficient and sufficient serum concentrations

Variable	≤50 nmol/L Mean ± SD	>50 nmol/L Mean ± SD	p-value
Age (years)	29.4 ± 5.6	30.1 ± 5.2	<b>0.03</b>
Weight (kg)	75.4 ± 13.3	74.1 ± 11.5	0.05
Height (cm)	162.8 ± 6.4	163.1 ± 6.0	0.42
BMI at OGTT (kg/m <sup>2</sup> )	28.5 ± 4.8	27.9 ± 4.0	<b>0.01</b>
Gestation (weeks)	29.1 ± 1.3	29.0 ± 1.2	0.23
Smoker in pregnancy			
Yes	296 (28.4%)	86 (15.9%)	<b>≤0.001</b>
No	747 (71.6%)	456 (84.1%)	
Alcohol use in pregnancy			
Yes	268 (25.7%)	426 (29.2%)	0.14
No	775 (74.3%)	1159 (70.8%)	
Education (years)	14.7 ± 2.8	15.3 ± 2.9	<b>≤0.001</b>
Season of sampling			
Summer & Autumn	416 (39.9%)	397 (73.2%)	<b>≤0.001</b>
Winter & Spring	627 (60.1%)	145 (26.8%)	
FH of diabetes			
Yes	167 (16.2%)	86 (15.9%)	0.87
No	865 (83.8%)	456 (84.1%)	
Fasting plasma glucose (mmol/L)	4.6 ± 0.3	4.6 ± 0.3	0.07
1-hour OGTT plasma glucose (mmol/L)	7.4 ± 1.6	7.4 ± 1.6	0.45
2-hour OGTT plasma glucose (mmol/L)	6.0 ± 1.1	6.0 ± 1.2	0.56
HOMA-IR	1.4 ± 1.4	1.4 ± 1.4	0.22
HOMA-beta	142.3 ± 1.3	141.9 ± 1.3	0.84
Diagnosis of GDM			
Yes	169 (16.2%)	74 (13.7%)	0.18
No	874 (83.8%)	468 (86.3%)	
Cholesterol (mmol/L)	6.4 ± 1.4	6.6 ± 1.4	0.08
HDL-cholesterol (mmol/L)	1.6 ± 0.4	1.7 ± 0.4	<b>≤0.001</b>
LDL-cholesterol (mmol/L)	3.8 ± 1.2	3.9 ± 1.2	0.32
Triglycerides (mmol/L)	2.1 ± 1.4	2.0 ± 1.4	0.71
Total 25OHD (nmol/L)	28.7 ± 11.5	80.1 ± 26.2	<b>≤0.001</b>
3-epi-25OHD <sub>3</sub> (nmol/L)	2.0 ± 1.1	4.5 ± 2.2	<b>≤0.001</b>
Dietary vitamin D (µg/day)	3.2 ± 2.3	3.5 ± 2.8	<b>0.01</b>

Continuous variable values are expressed as mean ± SD and categorical variables are expressed as number and percentage.

Gestational diabetes defined retrospectively by IADPSG/WHO criteria

HAPO, Hyperglycaemia and Adverse Pregnancy Outcome, BMI, body mass index OGTT, oral glucose tolerance test FH, Family history

Table 3- Unadjusted and adjusted associations of maternal 25OHD at an average of 28 weeks gestation with maternal glucose, beta-cell function, insulin resistance and lipid markers

Dependent Variable	Unadjusted				Adjusted <sup>b</sup>		
	n	Coefficient <sup>a</sup> (95% CI)	p-value	R <sup>2</sup>	Coefficient <sup>a</sup> (95% CI)	p-value	R <sup>2c</sup>
Fasting plasma glucose (mmol/L)	1562	-0.019 (-0.036 to -0.002)	<b>0.028</b>	0.00	-0.017 (-0.034 to 0.001)	0.06	0.13
One-hour plasma glucose (mmol/L)	1561	-0.008 (-0.091 to 0.075)	0.85	0.00	0.036 (-0.051 to 0.124)	0.42	0.09
Two-hour plasma glucose (mmol/L)	1562	0.022 (-0.037 to 0.082)	0.46	0.00	0.020 (-0.042 to 0.083)	0.52	0.09
HOMA-beta <sup>d</sup>	1546	1.006 (0.994 to 1.017)	0.33	0.00	1.013 (1.001 to 1.024)	<b>0.031</b>	0.21
HOMA-IR <sup>d</sup>	1546	0.996 (0.977 to 1.015)	0.69	0.00	1.007 (0.990 to 1.025)	0.43	0.32
GDM	1562	0.963 (0.835 to 1.111)	0.61	0.00	1.029 (0.872 to 1.216)	0.85	0.11
Triglycerides <sup>d</sup> (mmol/L)	1457	0.992 (0.973 to 1.011)	0.40	0.00	1.015 (0.994 to 1.035)	0.17	0.09
Cholesterol (mmol/L)	1457	0.062 (-0.014 to 0.139)	0.11	0.00	0.085 (0.002 to 0.167)	<b>0.044</b>	0.04
LDL (mmol/L)	1431	0.012 (-0.053 to 0.077)	0.72	0.00	0.023 (-0.047 to 0.093)	0.52	0.04
HDL (mmol/L)	1457	0.053 (0.029 to 0.077)	<b>&lt;0.001</b>	0.01	0.047 (0.021 to 0.073)	<b>&lt;0.001</b>	0.06

a- Regression coefficients represent the additive effect on the dependent variable associated with a doubling in maternal serum 25OHD level

b-Adjusted for season of sampling, maternal BMI at OGTT, maternal age, smoker during pregnancy, maternal education, maternal height, gestational age at OGTT and family history of diabetes as analysed by multiple linear regression.

c-R<sup>2</sup> value represents the entire adjusted model.

d- Regression co-efficient back transformed ( $2^{\wedge}$  (regression coefficient)) due to the dependent variable being log transformed.

Gestational diabetes defined retrospectively by IADPSG/WHO criteria.

25OHD, 25-hydroxyvitamin D GDM. Gestational diabetes mellitus BMI, body mass index OGTT, oral glucose tolerance test